Ph.D. THESIS

Effect of PARP inhibition on cuprizone-induced pathological changes in a rodent model of multiple sclerosis

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ABBREVIATIONS

4HQ: 4-hydroxiquinazoline
AIF: apoptosis inducing factor
CA II: carbonic anhydrase II
DAB: 3,3'-diamino-benzidine
DAPI: 4',6'-diamidino-2-phenylindole
DN: double negative
DP: double positive
EpCAM: epithelial cell adhesion molecule
EAE: experimental autoimmune encephalomyelitis
ERK1/2: extracellular signal-regulated kinase 1/2
FITC: fluorescein isothyocyanate
JNK: c-Jun N-terminal kinase
MAPK: mitogen activated protein kinase
MBP: myelin basic protein
MHC: major histocompatibility complex
MRI: magnetic resonance imaging
p38: p38 mitogen activated protein kinase
PAR: poly(ADP-ribose)
PARP: poly(ADP-ribose) polymerase
PBS: phosphate buffered saline
PE: phycoerythrin
PFA: paraformaldehyde
PI: propidium iodide
PI-3K: phosphatidylinositol-3 kinase
PKB/Akt: protein kinase B/Akt
ROS: reactive oxygen species
SP: single positive
TBS: Tris buffered saline
TEC: thymic epithelial cell

1. INTRODUCTION

1.1 Multiple sclerosis

Multiple sclerosis (MS) is a chronic disease of the central nervous system characterized by a presumed autoimmune inflammation, focal demyelination and axonal degeneration. Individuals with MS are usually young adults, with a prevalence of about 60-100:100.000 in Hungary. Environmental exposure and genetic susceptibility are both implicated as the cause of MS, although disease mechanism and pathogenesis are still under debate.

MS is a heterogeneous disease with various clinical symptoms and clinical course, moreover, there is profound heterogeneity also in pathological pattern of demyelination. Although all pathological patterns show a T cell- and macrophage-dominated inflammatory reaction, pattern I and II type demyelination is mainly immune-mediated (T cell/macrophage and B cell/complement, respectively), while in the earliest lesions of pathological types of pattern III and IV apoptosis-like depletion of oligodendrocytes have been described suggesting degenerative processes. An alternative hypothesis even proposes that oligodendrocyte apoptosis represents the first and earliest stage of all lesions resulting in secondary autoimmune inflammation.

Although immunomodulatory treatments are available to counteract the common inflammatory pathology, no treatments exist to prevent demyelination, which may contribute to axonal degeneration, the best pathological correlate of clinical symptoms in MS.

1.2 Cuprizone model of multiple sclerosis

A non-inflammatory experimental primary demyelination induced by the copper chelator cuprizone in young adult mice results in multi-focal demyelination and apoptosis of oligodendrocytes in particular brain areas, mainly corpus callosum. Mitochondrial aetiology was assumed since giant mitochondria have been observed in the liver and oligodendrocytes of cuprizone-treated mice. Supporting this notion, increased reactive oxygen species (ROS) production and decreased activities of the various complexes of the respiratory chain were found in mitochondria of cuprizone-treated oligodendroglia cells. However, and in contrast to the purely autoimmune experimental autoimmune encephalomyelitis (EAE), induced by immunization with myelin epitope, the number of T cells is negligible in the demyelinated corpus callosum and T cell activation has not been observed in the cuprizone model.

Besides demyelination and oligodendrocyte loss, administration of cuprizone to mice results in formation of hydrocephalus and weight loss of the animals too.

1.3 Poly(ADP-ribose) polymerase

Impaired functioning of the mitochondrial respiratory chain results in excessive production of ROS that induces damage to various cellular components including the DNA. The nuclear enzyme poly(ADP-ribose) polymerase (PARP) functions as a DNA damage sensor and signalling molecule. Activated PARP uses nicotinamide adenine

dinucleotide (NAD^+) as substrate and forms long branches of ADP-ribose polymers on a number of nuclear target proteins including PARP itself.

Extensive DNA damage triggers overactivation of PARP eventually resulting in cell dysfunction and death. According to a common hypothesis, overactivation of PARP and (ADP-ribosyl)ation leads to massive utilization of NAD^+. Loss of cellular NAD^+ and concomitant loss of ATP affects cellular energy metabolism resulting, ultimately, in cell death. Additionally, PARP activity appears to trigger mitochondrial outer membrane permeabilization and the mitochondria-to-nucleus translocation of apoptosis-inducing factor (AIF) resulting in chromatin condensation, fragmentation of DNA and cell death.

This nuclear mitochondrial crosstalk is supposed to involve PARP-dependent activity modulation of different kinase signalling pathways. Activated by the insulin/growth factor-phosphatidylinositol 3-kinase (PI-3K) cascade, protein kinase B (PKB), also known as Akt can prevent apoptosis through different mechanisms like inhibiting AIF translocation. It has been shown that PARP activity attenuates the cytoprotective PI-3K-Akt pathway. Moreover, it has been suggested that PARP activation leads to activation of the c-Jun N-terminal kinase (JNK) member of the mitogen activated protein kinase (MAPK) family, which leads, in turn, to mitochondrial depolarization, AIF translocation and cell death.

Furthermore, PARP has been shown to function as a co-activator in the nuclear factor (NF)-κB-mediated transcription regulating the expression of various pro-inflammatory proteins, i.e. inducible nitric oxide synthase, matrix metalloproteinase-9, etc.

PARP mediated cell death and inflammation has been implicated in the pathogenesis of several neurodegenerative diseases. Moreover, inhibition of PARP activity was able to ameliorate inflammation in EAE, indicating its central role in the mediation of autoimmune inflammation.

1.4 Immunological aspects of the cuprizone model

It has been described in EAE, that administration of cuprizone beginning one week prior to the injection of the encephalitogen attenuated disease severity. The mechanisms of disease suppression could be related to an inhibition of T-cell function since EAE involves stimulation of T-cell mediated immunity.

T cells, together with B cells, are the main cellular components of the adaptive immune system. T cells originate from the bone marrow, they migrate as immature precursor T cells into the thymus where they go through a series of maturation and selection steps thus establishing central self-tolerance. Most immature thymocytes express neither CD4 nor CD8 accessory molecules, they are $CD4^-CD8^-$ double negative (DN) cells. After acquisition of the CD3/T cell receptor complex and CD4, CD8 markers they become $CD4^+CD8^+$ double positive (DP) cells. In the thymic cortex they meet self-antigens presented on major histocompatibility complexes (MHC), where only those thymocytes will survive who bind the MHC/antigen complex with adequate affinity (positive selection). After becoming $CD4^+$ or $CD8^+$ single positive

(SP) thymocytes are again presented various self-antigens in the medulla, where thymocytes that interact too strongly with the antigen/MHC complex die by apoptosis (negative selection). Mature T cells then migrate to peripheral lymphoid organs to differentiate into effector cells and fulfil their function, where upon activation, cytotoxic T cells ($CD8^+$) eliminate foreign peptide presenting cells, helper T cells ($CD4^+$) secrete cytokins that regulate or assist in active immune response.

The thymic stroma provides the molecular and stromal environment for the development of T cells composed of thymic epithelial cells (TEC), dendritic cells, macrophages, fibroblasts and extracellular matrix components. In addition to the key antigen presenting interaction, they induce the complex programme of T cell maturation and modulate thymocyte differentiation. TECs are the major component of the thymic stroma and phenotypically distinct TEC subsets fulfil different functions during T cell development. Therefore, it can be distinguished between cortical epithelial cells that express Ly51 and EpCAM markers, mediating commitment, expansion, development of T cell presursors and positive selection in the cortex and medullary epithelial cells (EpCAM++, Ly51-) playing important role in negative selection mediation and providing survival signals to SP thymocytes.

It is important however, that the interaction between thymocytes and thymic epithelial cells is bidirectional, T cells are required for TEC development and formation of the three dimensional stromal structure. Therefore thymocytes and TECs exist in a dynamic co-dependence called cross-talk, whereby disruption of thymocyte development impacts on the stroma and vica versa.

2. OBJECTIVES

Administration of cuprizone to young adult male mice induces selective oligodendrocyte apoptosis with concomitant demyelination, formation of hydrocephalus and weight loss. Activation of PARP enzyme is involved in cell death processes in many pathological settings and PARP inhibition has been shown to act cytoprotective in many disease models of the CNS. Therefore, our first aim was to investigate,

1. whether PARP is activated upon cuprizone treatment in the corpus callosum and

2. whether its inhibition has any influence on cuprizone-induced demyelination, hydrocephalus formation and weight loss.

Cuprizone acts as a mitochondrial toxin, leading to impaired mitochondrial functioning and apoptosis of oligodendrocytes. However, the underlying molecular mechanisms of the cell death are not yet known. The aim of the next part of the study was therefore

3. to elucidate the possible mechanisms, signalling pathways involved in oligodendrocyte loss and

4. to investigate the effect of PARP inhibition on these processes.

It has been described previously that in EAE cuprizone exerted a disease attenuating effect, and modified peripheral T cell response. However, it is not known, whether there are any pathological changes of the thymus in the background of this phenomenon. Accordingly, the aim of the last part of the study was

5. to study the effects of cuprizone on thymi of cuprizone-treated mice,

6. and to investigate, whether PARP inhibition can modulate the assumed effect of cuprizone on the thymi of mice.

3. MATERIALS AND METHODS

Cuprizone demyelination model. Staring at 4 or 8 weeks of age, C57BL/6 male mice received a diet containing 0.2 m/m% cuprizone for one, 3, 5 or 6 weeks. The PARP inhibitor 4-hydroxyquiinazoline (4HQ, Sigma) was administered daily in a dose of 100mg/kg i.p. In order to follow the systemic effect of cuprizone, mouse weights were recorded every week of the treatment.

Thymocyte preparation and apoptosis detection. After one week of treatment animals were sacrificed, thymi were measured then homogenized mechanically and the suspension was filtered through a nylon mesh. Cell counts were determined by trypan blue exclusion method. For apoptosis detection, in AnnexinV binding buffer AnnexinV-FITC (BD Pharmingen) and propidium iodide (PI, Sigma) double staining then flow cytometric analysis was performed.

Thymocyte fluorescent labelling, flow cytometry. Anti-CD4, anti-CD8 and anti-CD3 (BD Pharmingen) labelling was performed for 30 min in binding buffer on ice, then cells were washed with PBS and resuspended in FACSFix buffer (1% paraformaldehyde in PBS) and analysed by flow cytometry. Flow cytometric analysis was performed using CellQuest software, 10.000 events were recorded, cellular debris and dead cells were excluded from analysis based on their morphology.

Immunfluorescence on thymus sections. After one week of treatment frozen tissue sections (7-10 μm thick) were prepared from removed thymi, then fixed with acetone, blocked in PBS containing 5% BSA and then sections were stained with anti-CD4 (FITC), anti-CD8 (Alexa fluor 647), anti-Ly51 (PE) and anti-EpCAM (FITC) antibodies (BD Biosciences and Department of Immunology and Biotechnology, University of Pécs). Sections were analysed using an Olympus fluorescent microscope.

Animal MRI and quantitative neuroimaging. Mice were anaesthetized weekly by injection of diazepam and ketamine, then measurements were performed using a Varian INOVA 400 WB NMR spectrometer (Varian Inc.) with a 9.4T magnet. Coronal images were recorded in a T_2-weighted multislice spin echo experiment obtaining 21 contiguous slices, each 1 mm thick. Mean signal intensities of the corpus callosum were standardized to the signal intensities of a reference capillary filled with a mixture of water/glycerine placed next to the animals. The extent of hydrocephalus was quantified calculating ventricle volume/ brain volume ratios.

Corpus callosum histology and immunohistochemistry. After 5 weeks of treatment mice were transcardially perfused and fixed with 4% PFA in PBS. Brains were dissected and embedded in paraffin then 8 μm thick coronal slices were obtained. Myelination of the corpus callosum was evaluated by Luxol fast blue (LFB)

staining. For immunohistochemistry, slides were blocked with 2% horse serum in PBS, then incubated with anti-PAR (Alexis) thereafter with appropriate biotinylated secondary antibody (Molecular Probes), DAB reaction was used for visualization, cells were counterstained with hematoxylin.

Corpus callosum immunofluorescence and confocal microscopy. For the evaluation of PARP activation (PAR formation) and AIF nuclear translocation fluorescent immunohistochemistry was performed after 3 weeks of treatment on 8 μm thick paraffin sections. Sections were stained with anti-PAR, anti-AIF (both Alexis) and anti-CAII (The Bindig Site) primary, then with fluorescent labelled appropriate secondary antibodies (Molecular Probes, Amersham, Jackson ImmunoResearch). Staining was finished with PI or DAPI (both Sigma) nuclear counterstain. Sections were examined with Olympus Fluoview confocal laser scanning microscope.

Immunoblot and densitometry. After 3 or 5 weeks of treatment corpus callosum of mice was dissected carefully and homogenized in the presence of protease inhibitor and sodium vanadate (both Sigma). Homogenates were boiled in sample buffer containing mercaptoethanol and SDS, subjected to electrophoresis and blotted onto nitrocellulose membrane. After blocking, membranes were incubated with anti-MBP (Novocastra), anti-PAR (Alexis), anti-AIF (Santa Cruz Biotechnology), anti-phospho-Akt (R&D Systems), anti-Akt/PKB, anti-phospho-ERK, anti-foszfo-JNK, anti-caspase-3 (all Cell Signaling), anti-phospho-p38-MAPK and anti-actin (both Sigma) antibodies, then with appropriate horseraddish peroxidase conjugated secondary antibodies (all Sigma). Visualization was performed by enhanced chemiluminescence, quantification was carried out using the Image J software (Bethesda).

Caspase-3 activity assay. After 3 weeks of treatment carefully dissected corpus callosum samples were homogenized in lysis buffer containing protease inhibitor cocktail. Fluorometric assays were performed using fluorescent-labelled peptide substrate for caspase-3 (Ac-DEVD-AFC, Sigma) on Fluostar optima fluorescence plate reader.

Statistics. For comparing the mass and cell count changes of thymi between the different treatment groups random effect analysis of variance, by the evaluation of flow cytometric measurements Student’s t-test was performed.
The time series of body weight and relative ventricle volumes measurements were analysed using a linear model, relative corpus callosum MRI signal intensities in the treatment groups were compared with a mixed effect analysis of variance. Immunoblot band intensities were normalized to the loading control and compared pairwise using Scheffé's post hoc ANOVA test. Evaluation was carried out using the SPSS and R software packages. Differences were considered significant at values of $p<0.05$.

4. RESULTS AND DISCUSSION

4.1 In experimental demyelination we examined activation of PARP by using an antibody produced against poly(ADP-ribose) (PAR), the enzyme's product in corpus callosum of mice after three weeks of treatment when the number of dying oligodendrocytes is the highest. By using anti-PAR immunoblotting we demonstrated significantly enhanced PARP activity in isolated corpus callosum and by using anti-PAR immunohistochemistry we observed strong PAR positivity in fragmented nucleus and cytoplasm of dying oligodendrocytes upon cuprizone treatment. Double staining and confocal laser-scanning microscopy revealed that the majority of cells with strong PAR immunoreactivity also expressed the oligodendrocyte marker CAII, confirming that elevated PARP activity originated from dying oligodendrocytes.

Therefore our data indicate that in agreement with our assumption –based on the observed mitochondrial pathology and apoptotic events– PARP activation occurred indeed in dying oligodendrocytes.

4.2 4-hydroxyquinazoline (4HQ), an inhibitor of PARP, blocked effectively both cuprizone induced PARP activity in demyelinating corpus callosum and basal PARP activity in corpus callosum of 4HQ only treated mice.

Parallel to PARP inhibition, 4HQ prevented the systemic effect of cuprizone, weight loss. As indicated by *in vivo* serial quantitative MRI, 4HQ treatment also prevented cuprizone-induced hydrocephalus formation.

In vivo serial MRI revealed also prevention of central nervous system demyelination by simultaneous 4HQ administration. Upon cuprizone feeding quantitative neuroimaging showed significant demyelination after 3 weeks up to 6 weeks, which was the most pronounced after 4 weeks of treatment and decreased thereafter because of spontaneous demyelination. Accordingly, inhibition of PARP prevented demyelination at all time points showing the strongest protective effect after 4 weeks of treatment. Pathological analysis with LFB staining confirmed our *in vivo* data and revealed reduced cuprizone-induced demyelination upon 4HQ co-treatment. Myelin basic protein (MBP) is expressed by oligodendrocytes and its amount correlates well with the amount of myelin. Densitometric evaluation of anti-MBP immunoblots performed on dissected corpus callosum revealed significant increase of the decreased MBP levels after 5 weeks of simultaneous 4HQ treatment, indicating myelin protective effect of PARP inhibition.

Since inhibition of PARP protected against demyelination, it can be concluded that PARP activation plays a key role during cuprizone induced oligodendrocyte apoptosis. PARP inhibition prevented also hydrocephalus formation and weight loss. Assuming that both hydrocephalus and weight loss develops because of cuprizone-induced disturbed energy metabolism of the cells, the observed protection could be explained by the cellular energy loss diminishing effect of PARP inhibition.

4.3 Thereafter we investigated the underlying mechanisms of cuprizone-induced, PARP-mediated experimental demyelination and oligodendrocyte death. After three weeks of treatment we investigated first in dissected demyelinated corpus callosum the activation of the caspase cascade dependent apoptotic pathway by two different methods, using a fluorescent caspase-3 activity assay or detecting cleavage of pro-caspase-3 by immunoblotting. The results suggested caspase-independent apoptotic mechanisms. Besides, we observed nuclear translocation of the apoptosis inducing factor (AIF) by immunohistochemistry and found also significantly elevated AIF expression by immunoblotting upon cuprizone treatment.

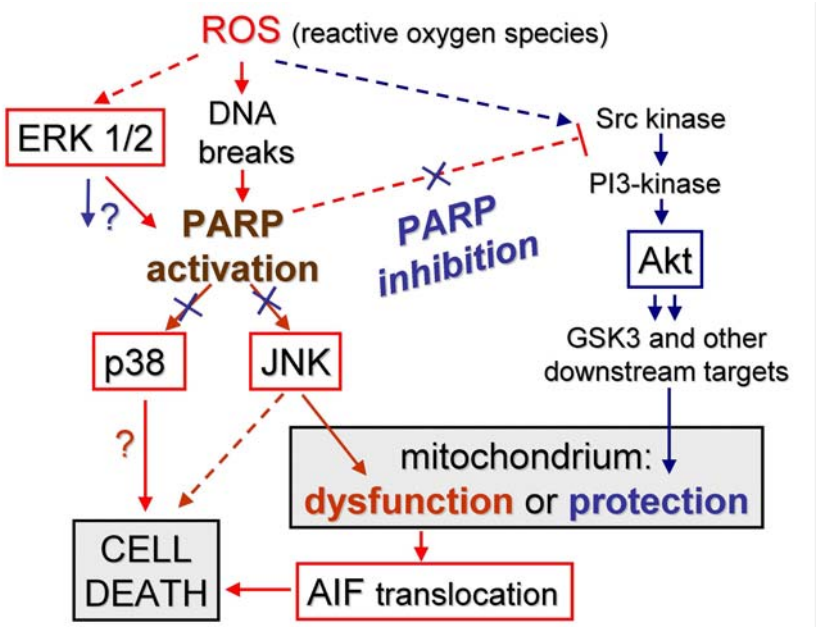

Fig. 1 Possible mechanisms underlying cuprizone-induced oligodendrocyte death and its prevention by PARP inhibition

Next, we examined in the corpus callosum the activity of MAPK and Akt cell signalling pathways that can be modified by PARP activity. Three weeks of cuprizone feeding resulted in elevated phosphorylation, i.e. activation of JNK, p38 MAPK and extracellular regulated kinase 1/2 (ERK1/2) MAP kinases, and also of Akt. JNK and p38 MAPK activation is considered to promote oligodendrocyte cell death, moreover, it has been suggested that activation of JNK is necessary for PARP induced AIF translocation. It has also been suggested that for a maximal PARP activation after DNA damage phosphorylation of the enzyme ERK is required. Therefore it seems likely that enhanced activity of MAP kinases contributes to PARP mediated cell death (fig. 1).

It is known that in response to numerous cell damaging effects Akt becomes activated and acts through different mechanisms against cell death. Our results may suggest similar events upon cuprizone intoxication, although it seems to be insufficient to protect oligodendrocytes.

4.4 Furthermore, we investigated mechanisms underlying prevention of demyelination by PARP inhibition. After three weeks of simultaneous cuprizone and 4HQ treatment neither elevated expression of AIF nor its nuclear translocation could be detected in the corpus callosum.

Moreover, 4HQ co-treatment decreased the activity of the pro-apoptotic JNK and p38 MAPK but did not affect activity of ERK supporting the notion that MAPKKK/ERK kinase/ERK1/2 pathway is upstream to PARP activation. PARP inhibition alone did not affect the phosphorylation level of MAP kinases.

Contrary to MAP kinases, simultaneous cuprizone and 4HQ treatment enhanced further Akt phosphorylation. It has been suggested that PARP activity blocks the activation of the PI-3K/Akt cytoprotective pathway, therefore PARP inhibition may release Akt from inhibition, activity of Akt increases resulting in cell protection. This can be supported by the observation that PARP inhibition alone increased Akt activation too (fig. 1).

4.5 In 4-week-old mice we observed already after one week of cuprizone treatment severe thymic atrophy with severe decrease of thymus mass and absolute thymocyte number. AnnexinV /PI double staining and flow cytometric analysis revealed that most of the thymocytes were early or late apoptotic upon cuprizone treatment, whereas the ratio of normal living cells decreased significantly.

Immunofluorescent microscopy showed the almost total disappearance of the CD4+CD8+ DP thymocytes in the cortex and a generally weak CD4 or CD8 immunopositivity of the thymus, which was in accordance with the great decrease of the total thymocyte number. CD4/CD8 double staining and flow cytometric analysis revealed a significant shift in the ratios of the different thymocyte populations. DP thymocytes, that gave about 75% of all thymocytes in control animals, disappeared almost totally accounting for only 1% of all living thymocytes upon cuprizone treatment. Concomitantly, the ratio of the other thymocyte populations increased to an approximately similar extent, indicating that –although the total thymocyte number and thus the number of cells in all populations decreased– the DP thymocyte population showed to be most vulnerable to the cuprizone challenge.

In order to investigate the effect of cuprizone on thymic stroma, Ly-51/EpCAM immunofluorescent staining was performed. Upon cuprizone treatment besides thymocyte loss a severe loss of thymic epithelial cells occurred too. A general decrease in cellularity affecting both cortical and medullary TECs was observed with disruption of the sponge-like structure of the stroma.

In summary, one week of cuprizone challenge resulted in severe thymic atrophy in 4-week-old mice. Similarly to many other pathological conditions, DP thymocytes, that are the most sensitive to different death stimuli, apoptosis inducing factors, proved to be again the most vulnerable. Besides thymocyte apoptosis, we also demonstrated severe damage of the thymic stroma resulting either from direct toxic effect of cuprizone or more possibly from the co-dependence between thymocytes and TECs.

Moreover, the observed changes of the thymus could explain –at least in part– the earlier described attenuated peripheral T cell response in EAE after cuprizone intoxication.

4.6 Co-treatment of 4-week-old mice with cuprizone and 4HQ resulted in significantly higher thymus mass, relative thymus mass and absolute thymocyte number compared to cuprizone only treated animals.

Although underlying mechanisms are completely unexplored, our data indicate that PARP inhibition could protect also against the thymic effects of cuprizone.

5. SUMMARY AND CONCLUSIONS

In our study we investigated an animal model of demyelination and oligodendrocyte depletion, as observed in multiple sclerosis. The mitochondrial toxin, cuprizone induces besides the selective apoptosis of oligodendrocytes also formation of hydrocephalus and weight loss in young adult C57BL/6 male mice.

We showed enhanced PARP activation in dying oligodendrocytes in the most demyelinated brain area, corpus callosum upon cuprizone treatment.

Administration of 4HQ, a potent PARP inhibitor decreased PARP activity and prevented demyelination. PARP inhibition protected mice also from development of hydrocephalus and severe weight loss.

We also intended to elucidate the underlying molecular mechanisms of cuprizone induced oligodendrocyte apoptosis and found besides PARP activation elevated AIF expression, AIF nuclear translocation and revealed no caspase-3 activation. We detected increased phosphorylation levels of the MAP kinases, i.e. ERK1/2, p38 MAPK, and JNK and of the cytoprotective Akt/PKB upon cuprizone treatment in the corpus callosum. PARP inhibitor co-treatment decreased elevated AIF expression, prevented AIF nuclear translocation and attenuated the activation of JNK and p38, which are usually known to play a role in apoptosis and inflammation. Besides, PARP inhibition enhanced further the phosphorylation of Akt contributing to the cell protecting effect of 4HQ.

Based on these data and on our previous observations about pattern III MS, it could be assumed that besides the morphological similarities, cuprizone induced demyelination and pattern III MS share at least two key molecular mechanisms: PARP activation and AIF-mediated caspase-independent apoptosis. Since PARP inhibition has a direct effect on inflammation indicated by reduced clinical signs of EAE, inhibiting PARP thus may provide the first therapy influencing effectively all pathological types of MS by protecting from both autoimmune inflammation and degenerative oligodendrocyte death.

Besides the already known effects of cuprizone, we described at the first time that cuprizone administration to 4-week-old male mice resulted in severe thymic atrophy even after one week. We demonstrated that thymocytes died by apoptosis and found $CD4^+$ $CD8^+$ DP thymocytes the most vulnerable. We demonstrated also the disruption of the thymic stroma.

Moreover, we showed that PARP inhibition protected mice from acute thymic involution. Acute thymic involution and concomitant impaired peripheral immune response is a complication of malnutrition, infections, sepsis or other stress events. Since PARP inhibition showed promising results in preventing cuprizone induced thymic atrophy and as there are currently no treatments available to protect against acute thymic involution or accelerate recovery leaving the immune system compromised during acute stress, further investigations in this topic could be of great importance.

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7. PUBLICATIONS

Publications supporting the thesis:

Veto S*, Acs P*, Bauer J, Lassmann H, Berente Z, Setalo G Jr., Borgulya G, Sumegi B, Komoly S, Gallyas F Jr, Illes Z. Inhibiting poly(ADP-ribose) polymerase: a potential therapy against oligodendrocyte death. Brain, 2010, 133:822-834 IF:9.49
**SV and PA contributed equally*

Manuscript in preparation: New aspects of the degenerative demyelination model – acute thymic atrophy induced by cuprizone.

Other publications:

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Patent:

Pharmaceutical combination product for the treatment of degenerative neurological diseases. Completed Hungarian application No.: P0800414, PCT/HU2009/000055 Priority date: 2008. 07. 04.

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